



Eur päisches
Patentamt

European
Patent Office

Office eur péen
des brevets



Bescheinigung

Certificate

Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

01200493.3

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

DEN HAAG, DEN
THE HAGUE,
LA HAYE, LE
24/01/02



BEST AVAILABLE COPY



Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

**Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation**

Anmeldung Nr.:
Application no.: **01200493.3**
Demande n°:

Anmeldetag:
Date of filing:
Date de dépôt: **09/02/01**

Anmelder:
Applicant(s):
Demandeur(s):
UNILEVER N.V.
3013 AL Rotterdam
NETHERLANDS

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Food product comprising soy protein and statins

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: Tag: Aktenzeichen:
State: Date: File no.
Pays: Date: Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:
A23L1/29, A23L1/30, A23L1/305, A23L1/308, A61K35/78

Am Anmeldetag benannte Vertragstaaten:
Contracting states designated at date of filing: AT/B/E/C/H/C/Y/D/E/D/K/E/S/F/I/F/R/G/B/G/R/I/E/I/T/L/I/L/U/M/C/N/L/P/T/S/E/T/R
Etats contractants désignés lors du dépôt:

Bemerkungen:
Remarks:
Remarques:
See for title page 1 f the description

BEST AVAILABLE COPY

09 02 2001

Food product suitable for reducing low density lipoprotein cholesterol levels

44

Description

5

Technical field

The present invention relates to food products suitable for reducing low density lipoprotein cholesterol levels in the 10 human blood.

Background of the invention

Cardiovascular disease is a leading cause of morbidity and 15 mortality, particularly in the United States and in Western European countries and is emerging in developing countries. Several factors are mentioned relation to the development of cardiovascular disease including hereditary predisposition to the disease, gender, lifestyle factors such 20 as smoking and diet, age, hypertension, and hyperlipidemia, including hypercholesterolemia. Several of these factors, particularly hyperlipidemia and hypercholesterolemia, contribute to the development of atherosclerosis, a primary cause of vascular and heart disease.

25

Elevated low-density lipoprotein cholesterol (hereafter "LDL-cholesterol") is directly related to an increased risk of coronary heart disease.

30 The presence of soy protein in food consumed by humans is associated with a lower level of low-density lipoprotein cholesterol (LDL-cholesterol) and lower risk of coronary heart disease.

Food products comprising soy protein are well known. Examples of food products that may comprise soy protein are meat products, baked products, food analogs and dairy products. Types of soy proteins and food uses of soy proteins are 5 described in Waggle, D.H. and Kolar, C.W., Soy protein and human nutrition, proceedings of a symposium held May 222-25, 1978 in Keystone Colourado, pages 19-51.

Soy protein materials are known to reduce total cholesterol and 10 LDL-cholesterol levels in the blood of animals.

An analysis of the effects of soy protein intake on serum lipids in humans has shown that dietary soy protein is significantly related to lowering serum concentrations of total cholesterol and LDL-cholesterol in humans (Anderson, Johnstone, 15 and Cook-Newell, N. Engl. J. Med., Vol. 333, No. 5, pp. 276-82 (1995)).

Statins are compounds that are known to have a lowering effect on levels of LDL-cholesterol in the human blood. Statins 20 inhibit the hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-determining step in the cholesterol biosynthesis.

Scientific research has confirmed the healthy properties of 25 statins especially with respect to LDL blood-cholesterol and triglyceride levels lowering activities, both in animals and in humans (Li et al., Nutrition Research 18, 71-81 (1998); Heber et al., Am. J. Clin. Nutr. 69, 231-236 (1999)).

30 Early reports of the effect of statins were made in 1979. The Japanese scientist Endo isolated a metabolite from *Monascus* that reduced artificially induced hyperlipoproteinemia in rats (Endo, J: Antibiotics 32, 852-854, (1979)). These metabolites are known as monacolins. Monacolin is identical to the

cholesterol lowering pharmaceutical lovastatin. Lovastatin is sold by Merck co. under the tradename Mevacor. A derivative of lovastatin, simvastatin, is sold as a cholesterol-lowering drug under the name of Zocor. Other derivatives of lovastatin e.g. 5 pravastatin, and mevastatin, are also sold as lipid lowering drugs against hypercholesterolemia. *Monascus*-extracts are sold in capsules in Japan as the dietary product Monacolin. The usual dose of the above statins is 20 mg/day, which results in at least 20% blood LDL-cholesterol lowering.

10

The production of statins is also reported in fermentation using fungi other than the above-mentioned *Monascus* species. It has been shown that statins can be produced by a variety of filamentous fungi, including *Monascus*, *Aspergillus*, 15 *Penicillium*, *Pleurotus*, *Pythium*, *Hypomyces*, *Paelicilomyces*, *Eupenicillium*, and *Doratomyces*.

The preparation and purification of the statins used in pharmaceutical preparations involves many process-steps, in 20 which ingredients are used that are not commonly used in the food industry. The many process steps increase costs compared to processes having less process steps. For these reasons the statins prepared for pharmaceutical use are not used in the foods industry.

25

As a food product, rice fermented with a red *Monascus* fungus (red rice) has been known and used for hundreds of years in China. Red rice was used and still is used in wine making, as a food-colouring agent and as drug in traditional Chinese 30 medicine. We have found that most red rice available on the market contains no statins or statins in very low amounts. The Food and Drug Administration has concluded that red yeast rice available in the market does not contain significant amounts of lovastatin (FDA, Docket No. 97-0441, Final Decision).

WO 99/23996 describes a composition for treating elevated serum cholesterol and/or triglycerides comprising a red rice product containing at least 0.05% lovastatin by weight.

5

Red rice powder capsules are sold as dietary supplements under the name of Cholestin by the firm Pharmanex. Pharmanex also sells a Cholestin bar containing red yeast rice (*Monascus purperus went*).

10

Red rice has an intensive red colour. Whereas the intensive red colour of red rice is an advantage when it is used as colouring agent, it is a disadvantage when it is used in the majority of food products. Due to the intense red colour of red-rice products, the foods prepared from red rice are coloured, depending on the amount of red-rice product added to the food product yellow, orange or red. The higher the amount of red rice added to the food, the more intense is the red colour of the food product. In the known food products a relatively large amount of red rice has to be added in order to add enough statins. This results in a red colour of the products that cannot be avoided.

In some food products the red rice colouring is undesirable. In particular in the western world, consumers are reluctant to use products of which the colour has changed from that they are used to. For example spreads, including margarine, butter, low fat spreads and cooking or salad oils are considered unacceptable by customers when the colour of such a product is intense yellow or orange or red. However, at the same time these type of products have been found by the applicant to be excellent vehicles of the daily intake of amounts of statins sufficient to obtain a blood LDL-cholesterol lowering effect.

Summary of the invention

It is an object of the invention to provide a food product that has no undesired colouring due to the addition of statins. A 5 further object of the invention is to increase the health effects of the known food products comprising statin. Another object is to provide a process for the preparation of a food product comprising statin, which involves less process steps than in the preparation of statins as a pharmaceutical drug. 10 Another object is to provide such a process, which avoids the use of ingredients or process aids that are not commonly used in the food industry. One or more of these objects are attained by the invention.

15 We have now found a food product comprising an amount of soy protein of at least 5 grams per average serving, characterized in that the food product comprises at least 5 mg/kg statins.

Advantageously the food product comprises a fermented soy 20 ingredient.

Surprisingly we have found that when the substrate for the Monascus fermentation is soybeans and/or soybean ingredients, the red colouring of the fermented product as in red rice 25 fermentation is avoided, i.e. a non-coloured or only slightly coloured fermentation product is obtained. Further we have found that compounds having a positive health effect, which are present in soybeans are also present in the fermented product. These compounds include, but are not limited to polyunsaturated 30 fatty acids, phytosterols, dietary fibers including soluble fibers, polyphenols and saponins. As a result of the presence of these compounds in the fermentation product, the food product according to the invention has increased health effects compared to the known food products comprising statin.

Detailed description of the invention

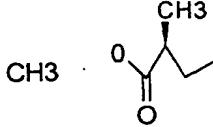
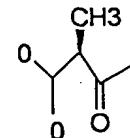
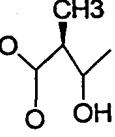
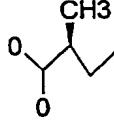
5 The following definitions will be used.

The amounts given will be expressed, in wt.% or weight parts per million (ppm), mg/kg or g/kg, relative to the total weight of the food product.

10

Statins are defined as substances having the structural formula, presented in figure 1. In this structural formula, R1 and R2 can be any group. Preferred statins are those that are given in table 1.

Table 1: Preferred statins according to formula of figure 1

	R1	R2	
monakolin K	CH3		
monakolin L	CH3	H	
monakolin J	CH3	OH	
monakolin X	CH3		
monakolin M	CH3		
compactin (ML-236B)	H		
ML-236-A	H	OH	
NL-236-C	H	H	

Food products according to the invention are preferably foods in which soy protein materials are used as functional ingredients.

5 They include, but are not limited to, meats such as ground meats, emulsified meats, fermented meats and marinated meats, beverages such as nutritional beverages, sports beverages, protein fortified beverages, juices, milk, milk alternatives, and weight loss beverages, cheeses and cheese like
10 products, such as tofu, frozen desserts such as ice cream, ice milk, low fat frozen desserts, and non-dairy frozen desserts, yoghurts, soups; puddings, breakfast cereals, pasta products, bakery products, such as bread and cake, salad dressings, and dips and spreads such as mayonnaise, chip dips, low fat
15 spreads, sandwich spreads, dietetic products e.g. slimming products or meal replacers etc.

The statins and soy proteins are present in the food product in an amount sufficient to obtain a blood LDL-cholesterol lowering
20 effect if the food product is used according to the common needs of the consumer.

The skilled person will be able to adjust the percentage of statins and soy proteins in the food product to get the above
25 effect. The percentages will depend on the type of food product, since the food products are used in different serving sizes. Moreover the pattern in a food product is consumed (servings per day and distribution over days) is dependent on the food product.

30

As an illustration table 1 indicates a number of products, which may be prepared according to the invention, and a typical serving size.

Table 1:

Product	Average Serving (grams)
Margarine	15
Meat product	50
Bar	60
Meal replacer drink	330
Yoghurt type drink	200
Ice cream	75
Cereal	50
Beverage	200

Preferably the food product according to the invention is a spread, meat product, bakery good, breakfast cereal, beverage or bar. These products are preferred because the way in which they are consumed results in a more controlled and constant intake of statins and soy proteins than in other food products. More preferred food products according to the invention are a spread, bar, or beverage.

The invention will now be further illustrated by the description of suitable embodiments of the more preferred food products. It belongs to the ability of the skilled person to use the teaching provided therewith to prepare other products of the invention.

- Meat products

Meat products according to the invention usually have a composition of known meat products, however part of the protein in the meat product is soy protein comprising statin. Examples of types of meat products are hamburgers, sausages, such as

fermented sausages, cooked sausages, such as frankfurter type sausages, pate, etc.

An example composition of a fermented sausage is: minced meat 5 40-70 wt.%, mycoprotein 0-25 wt.%, 5-30 wt% fermented soy protein comprising statin, 0.1-1 wt.% sodium caseinate, 0.1-1 wt.% transglutaminase, nitrite salt 0.5-5 wt.%, glucose 0.1-1 wt.%, *Pediococcus* culture mix 0.001-0.010 wt.% and 0-1 wt% flavouring, salt, etc.

10

An example composition of a frankfurter type sausage is: 10-40 wt.% minced pork meat, 5-20 wt.% minced beef meat, 5-25 wt.% fermented soy protein comprising statin, 0-3 wt.% salt, 0-2 wt.% nitrite, 0-3 wt.% phosphate, 20-50 wt.% water.

15

A typical size for a meat product could be from 20 to 200 g, generally from 40 to 100 g. Preferred levels of statins and protein in such products are: 15-350 mg/kg statin and 30-300 g/kg soy proteins. More preferred ranges for these levels are 50 to 20 200 mg/kg statin and 50-400 g/kg soy proteins respectively.

- Beverages

25 Preferred food products according to the invention are beverages, for example tea, fruit juice, soft drinks, meal-replacer drinks, etc.

The beverage may be a beverage which is ready to drink or a 30 powdered beverage, to which water has to be added to prepare a ready to drink beverage.

Meal replacer drinks will be described in more detail herein below. It will be apparent that similar levels and compositions

apply to other beverages comprising statins and soy proteins. A typical serving size of a beverage is taken to be 200 ml.

Meal replacer drinks are typically based on a liquid base which 5 may for example be thickened by means of gums or fibres and whereto a cocktails of minerals and vitamins are added. The drink can be flavoured to the desired taste e.g. fruit or chocolate flavour. A typical serving size may be 330 ml or 330 grams.

10 An example composition for a meal replacer beverage is about 70-85 wt.% water, 2-10 wt.% soy protein comprising statins, 5-15 wt.% sugar (e.g. sucrose), 0-4 wt% fat, 0.1-1 wt.% vitamins and minerals, 0.01-2 wt.% flavour, 0.1-0.5 wt.% thickener.

15 Preferred levels of statins and protein in a beverage are: 20-100 mg/kg statin and 20-80 g/kg soy proteins. More preferred ranges for these levels are 30 to 70 mg/kg statin and 30-50 g/kg soy proteins respectively.

20

- Food bars, including cereal bars

These products often comprise a matrix of edible material wherein the statin and soy proteins can be incorporated. For 25 example the matrix may be fat based (e.g. couverture or chocolate) or may be based on bakery products (bread, dough, cookies etc). Preferably the food product is a cereal bar, in which the matrix is based on agglomerated cereal particles (rice, grain, nuts, raisins, fruit particles).

30

The matrix material of a bar may be present in an amount of 60-95 wt.% of the weight of the bar, preferably 70-90 wt.% most preferred 75-85 wt.%: The matrix material may contain 5-30 wt.%

soy ingredient comprising statin, preferably 10-25 wt.%, more preferably 15-25 wt.%, based on total weight of the food bar.

Other ingredients in the cereal bar may be starch, sugar (e.g. 5 0-10 wt.%), sirups, honey, water, milk solids (0-10 wt.%), salt (e.g. 0-5 wt.%) calcium carbonate (e.g. 0-5 wt.%), vitamins, flavouring and colouring.

The ingredients are usually mixed and cooked (e.g. by cooking-10 extruding), formed to the desired shape and size, to produce the (cereal) bar.

A typical size for a food bar could be from 20 to 100 g, generally from 40 to 60 g. Preferred levels of statins and 15 protein in food bar are: 50-500 mg/kg statin and 50- 300 g/kg soy proteins. More preferred ranges for these levels are 100 to 350 mg/kg statin and 80-250 g/kg soy proteins respectively.

20 • Dairy type products

Examples of dairy products according to the invention are dairy spreads, cream cheese, milk type drinks and yoghurt, where the milk solids are partly or fully replaced by fermented soy ingredient, preferable fermented soy protein isolate.

25

An example of a composition for a yoghurt type product is about 50-80 wt.% water, 3-12 wt.% soy protein comprising statins, 0-15 wt.% whey powder, 0-15 wt.% sugar (e.g. sucrose), 0.01-1 wt.% 30 yoghurt culture, 0-15 wt.% fruit, 0.05-0.5 wt.% vitamins and minerals, 0-2 wt.% flavour, 0-5 wt.% stabilizer (thickener or gelling agent).

A typical serving size for a yoghurt type product could be from 125 to 250 g, generally from 150 to 225 g. Preferred levels of statins and soy protein in the yoghurt type product are: 40-200 mg/kg statin and 20-100 g/kg soy proteins. More preferred ranges for these levels are 40 to 80 mg/kg statin and 30-60 g/kg soy proteins respectively.

- Frozen Confectionery Products

10

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet; sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees.

15

Preferably the level of solids in the frozen confection (e.g. sugar, fat, flavouring etc) is more than 3 wt.%, more preferred from 10 to 70 wt.%, for example 40 to 70 wt.%.

20

Ice-cream will typically comprise 2 to 20 wt.% of fat, 2 to 20 wt.% of soy ingredient comprising statin, sweeteners, 0 to 10 wt.% of non-fat milk components and optional components such as emulsifiers, stabilisers, preservatives, flavouring ingredients, vitamins, minerals, etc, the balance being water. Typically ice-cream will be aerated e.g. to an overrun of 20 to 400 %, more specific 40 to 200 % and frozen to a temperature of from -2 to -200 °C, more specific -10 to -30 °C. Ice-cream normally comprises calcium at a level of about 0.1 wt%.

25

Other food product according to the invention can be prepared by the skilled person based on common general knowledge, using soy protein comprising statin as an ingredient in suitable amounts. Examples of such food products are baked goods, dairy type foods, snacks (e.g. roasted soynuts), etc.

Preparation of the food product

5 According to the invention a substrate, prepared from soybeans and/or ingredients thereof, such as defatted soy, soy protein concentrate or isolate, textured soy protein, etc. is fermented with a *Monascus* fungus and the fermentation product is used in the preparation of a food product. These steps will be
10 illustrated below.

Fermentation is conducted in known way. The fermentation is conducted in at least one fermentation vessel (fermenter) in which a medium comprising soybeans and/or ingredients thereof
15 is present. The fermentation is started (inoculated) by adding a suspension of spores of the *Monascus* fungus (inoculum), which has been prepared by fermenting *Monascus* fungus on a separate medium.

20 The fermentation involves the following steps, which are executed in the given order:

- a) Preparation of the medium for the inoculum and the medium to be used in the fermenter
- b) Sterilization of the media, fermenters and ancillary
25 equipment
- c) Production of inoculum
- d) Addition of the inoculum to the medium comprising soybeans and/or ingredients thereof, in the fermenter.
- e) Conducting the fermentation
- 30 f) Removal of the fermentation product from the fermenter

The fermentation product is used in the preparation of the food products according to the invention.

Optionally, before the fermentation product is used in the preparation of the food products, the following additional process steps may be executed:

- g) Sterilization of the fermentation product
 - h) Drying of the fermentation product (or sterilized fermentation product)
- 5 i) One or more separation steps, for instance extraction, to separate statins and soy actives from *Monascus* biomass in the fermentation product

The medium used in the fermenter may be solid or liquid.

- 10 Advantageously the medium is solid, most preferred the medium substantially consists of crushed whole soybeans, which have been soaked with water until the moisture content reaches 20-35 wt.% water). In case the medium is liquid, usually water is present as major constituent of the medium.

15

Whole soybean are preferably used as a substrate for the fermentation. Typical composition of whole soybeans is 42% wt.% protein, 20 wt.% oil, 35% wt.% total carbohydrates, 5 wt.% ash and 5.5 wt.% crude fiber (Kawamura, S., Tech. Bull. Faculty

20 Agric., Kagawa Univ., 18, 117 (1967)).

Instead of whole soybeans, parts or ingredients of soybean may be used in the medium for the fermenter, for instance soy protein (including textured vegetable protein), soy milk, soy-
25 flakes etc. Care has to be taken that the medium contains compounds that can provide a carbon source and a nitrogen source for growth of the *Monascus* fungus.

The *Monascus* fungus used according to the invention may be any 30 *Monascus* fungus that produces statins. Preferably the fungus is chosen from the group of *Monascus ruber*
Most preferred is *Monascus ruber* F125 M1-4.

Strains F125 and F125 M1-4 are deposited at the Centraal Bureau voor Schimmelculturen (CBS) as no. CBS 109070 on 14.11.2000 and no. CBS 109269 on 23.01.2001.

- 5 These deposit were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty).
- 10 Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.
- 15 The medium will ordinarily be sterilized before fermentation, e.g. by heat treatment, like pasteurization.

The medium in the fermenter may contain other substances, which may aid the fermentation, for instance sugars, amino acids and 20 vitamins.

The fermentation may be carried out in a manner, which can be determined by the skilled person on the basis of common general knowledge of fermentation technology. As illustration preferred 25 embodiments are described hereunder.

The fermentation temperature may be important. The temperature is preferably in the range of 10 to 37°C more preferably 20 to 30°C. We have found that at 37°C and higher the production of 30 statins decreases.

Preferably during fermentation the medium is aerated, e.g. by stirring, shaking etc. Aeration may be carried out by blowing air through the fermentation medium. Preferably the air is

wholly or partly saturated with water vapour in case solid state fermentation is used. This avoids drying out of the fermentation medium.

5 The relative levels of statins to soy actives will depend on the fermentation time. The fermentation time is therefore dependent on the desired amount of statins in the fermentation product. Preferred fermentation time is 1-50 days, more preferably 15-40 days, most preferably 20-30 days.

10

Prior to addition to the food product, the fermentation product may be subjected to a separation step, to separate statins and soy actives from *Monascus* biomass in the fermentation product. This separation may be done with known separation techniques, 15 e.g. filtration or centrifugation.

The fermentation product may also be extracted and the extract may be used in the preparation of the food product.

20 The extraction may be done on the fermentation product. Alternatively the *Monascus* biomass may be separated from the fermentation product prior to extraction, e.g. by filtration. The *Monascus* biomass may be separately extracted and the resulting extract can also be used in the preparation of the 25 food product.

Extracts may be used as such in the preparation of food products. Preferably extraction solvent may be removed from the extracts, e.g. by evaporation of the extraction solvent.

30 Alternatively the fermentation product may be diluted, e.g. by adding soy ingredients.

The fermentation product (including extracts etc.) may directly be added to food product ingredients in the process of

preparation of the food products according to the invention. It, however may be required that the fermented product is pasteurized, dried, etc. in order to preserve or modify it before it is further used for the preparation of a food product. It can be added to the other ingredients of the food product composition, or it may be added to part of the ingredients, before other ingredients are added. If more than one phase is present in the food product the fermentation product may be present in one or more of these phases.

10 Preferably the fermentation product will substantially be present in an oil phase, if such oil phase is present.

Soy protein content in a food product according to the invention may be measured according to the method described in
15 Agater et al., J. Sci. Food Agric. (1986), 37(3), 317-31.

The invention will be further illustrated in the examples.

Examples

20

Example 1

A. Preparation of *Monascus* strain F125 M1-4

25

Monascus ruber strain F125 was cultivated in malt water liquid medium at 30°C for 4 days. Of this culture, 1 ml was used as an inoculum for a Hybond-N filter (Amersham, UK) placed on a YE plate (4% glucose, 0.3% KH₂PO₄, 1.0% yeast extract (Difco), 1.5% agar). After 3 days incubation at 30°C, the spores were harvested by washing the filters with 10 ml physiological saline containing 0.1% Tween 80. The spores were filtered 4 times through Mira cloth filters to obtain a hyphae free spore

suspension. This suspension was used for subsequent mutagenesis.

The spores were diluted to a concentration of 10^8 spore/ml then 5 exposed to UV light at an intensity of 100 joules/m². The mutagenised spores were plated on Potato Dextrose Agar (Oxoid) and incubated for 3 weeks. One of the resulting colonies, which had a lighter colour than the others was selected and is herein defined as *Monascus* strain F125 M1-4.

10

The strain F125 M1-4 was deposited at the Centraal Bureau voor Schimmelculturen (CBS) on 14.11.2000 and has number CBS 109070.

B. Preparation of *Monascus* spores

15 Monascus f125M1-4 spores were prepared by harvesting *Monascus* mycelium from a PDA (potato dextrose agar) (oxoid) slope by washing with 5 ml physiological saline and incubating the mycelium in 150 ml malt water (oxoid) for 4 days at 30°C. The spores were harvested by filtration through a Mira cloth
20 filter.

C. Fermentation

C.1. Preparation of an inoculum

A shake flask containing textured vegetable protein (TVP) is 25 inoculated with filamentous spores suspended in a physiological water solution containing 0.1 wt.% Tween 80 (polyoxyethylene sorbitan fatty acid ester, available from ICI Specialty Chemicals™). This shake flask is incubated to let the mould grow. This results in a *Monascus* spore suspension, which was 30 adjusted by dilution to a concentration of 1×10^6 spores/ml.

C.2. Preparation of fermentation medium

1 kg of TVP is soaked in tap water (50°C) for 30 minutes. After soaking the TVP bits are rinsed with cold tap water.

Subsequently 50 g batches of the soaked TVP (35 wt% dry matter) were brought in to 300 ml Erlenmeyer flasks.

C.3. Fermentation

- 5 The shake flasks were each inoculated with 1 ml of the prepared *Monascus* spore suspension and incubated for 30 days at 30 °C. A sample from the shake flask was taken every week to monitor the statin production.
- 10 After fermentation, about 600 g textured Soy protein (moisture content 28%) remained. The fermented soy ingredient was pasteurized for 85 °C for 30 mins, cooled and stored for the preparation of food product.

15

D. Statin analysis of fermentation product

The fermentation product samples are extracted in a 50 ml tube (Falcon) by adding 6 ml of a mixture of acetonitril, water and 20 phosphoric acid (1:1:0.05, v/v/v)

The mixture is blended with an Ultratorax for 1 min. The mixture was then incubated at room temperature on a rollerbank for over 24 hours. Hereafter the samples were centrifuged and the supernatant liquid used for HPLC analysis. Samples were 25 separated using HPLC analysis on a Shimadzu apparatus according to the method of Morovjan et al., J. chromatogr. A 763 (1997) 165-172. The system consists of the Shimadzu SCL-10A system controller, CTO-10AS column oven, LC-10AT vp pump system, RID-10A refraction index detector, SPD-M10A diode array detector 30 and SIL-10AD autoinjector. For the chromatographic determination of statins a Waters NovaPak C18 (150x3.9 mm I.D., 4µm) column was used operating at 25°C. The eluent was acetonitril-0.1% phosphoric acid (50:50, v/v) solution flowing at 1.5 ml/min. Runs were performed for 15 min. The detection

was performed using a diode array detector from 190 nm up to 800 nm. The sum of the area of all peaks in the spectrum belonging to statins is measured. Comparison to a standard (Mevinolin, Sigma) allows the calculation of a statin content 5 (expressed in mg/kg analysed product).

The analysis results in a statin content of 1200 mg/kg.

E. Preparation of a fermented sausage

10

Fermented sausages having the following composition were prepared:

Table 1: Composition of fermented sausage

15

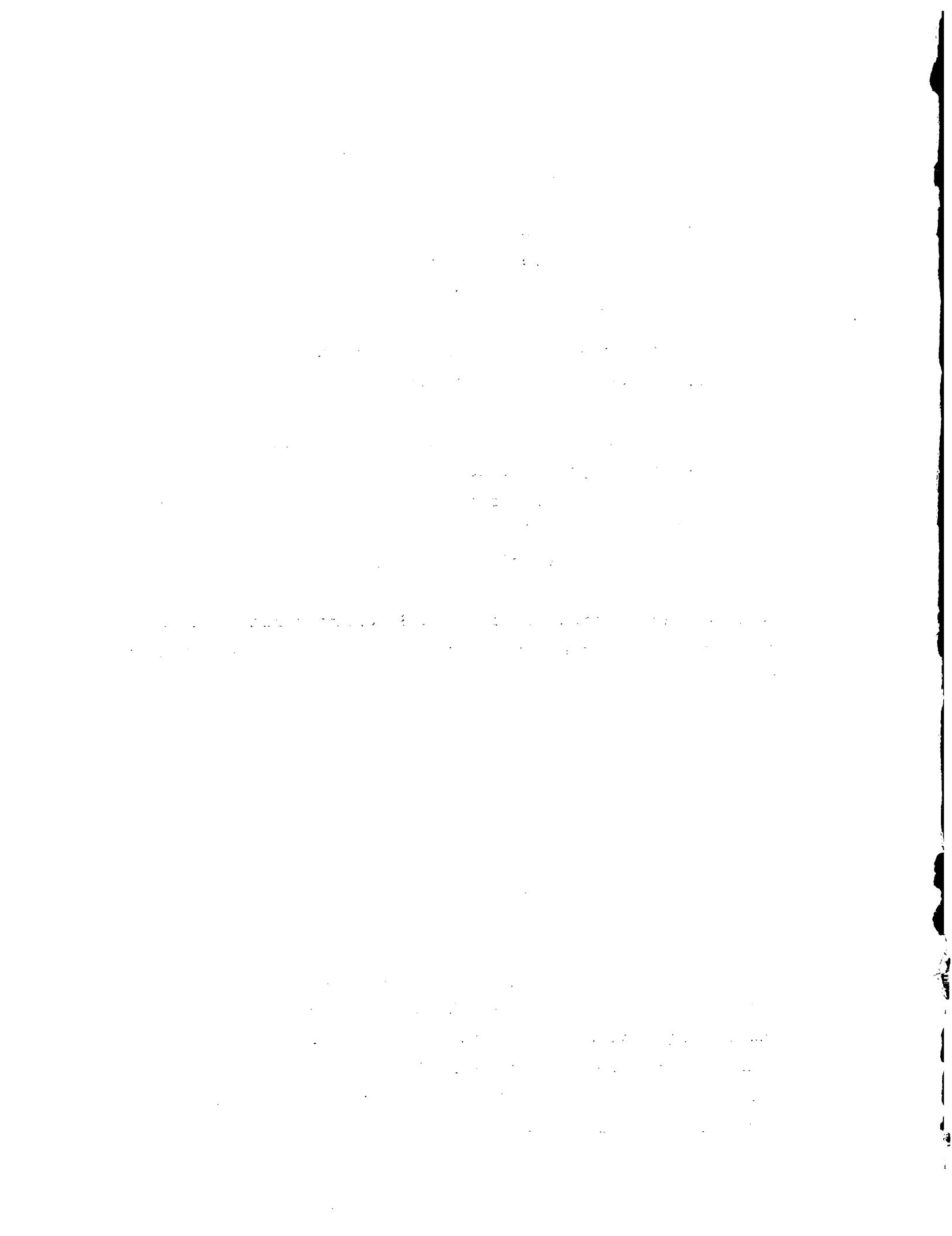
Ingredient	Wt.% sample a	Wt.% sample b
Monascus fermented TVP	10	20
Quorn (mycoprotein based meat replacer, ex Marlow foods Limited)	25	15
Salted minced pork	35.6	35.6
Salted bacon	25	25
Na-caseinate	0.4	0.4
Trasglutaminase Active	0.4	0.4
Nitrite salt	1.96	1.96
Sodium ascorbate	0.06	0.06
Glucose	0.4	0.4
Pediococcus starter culture	0.016	0.016

from Quest Int.		
Water	0.024	0.024
Total	100	100

The fermented sausages were prepared by mixing all ingredients.
The resulting mixture was fed into sausage casings and the
5 casings were stored for 24 hours at 2°C.

Then, the fermentation of the sausages was executed at 28°C for
about 24 hours, until the pH was 5.2. The resulting sausages
were dried at 20°C for 4 days. The pH of the sausages was 4.7
10 and the dry matter content 72 wt%.

The fermented sausage contained 65g/kg (sample a), respectively
135g/kg (sample b) soy protein and 110 mg/kg (sample a),
respectively 245 mg/kg (sample b) statins as analysed by the
15 method described hereinbefore.



EPO - DG 1

24

09 02 2001

Claims

(44)

1. Food product comprising an amount of soy protein of at least 5 grams per average serving, characterized in that the food product comprises at least 5 mg/kg statins.
2. Food product according to claim 1, characterized in that the food product comprises a fermented soy ingredient.
3. Food product according to claim 2, characterized in that the fermented soy ingredient is the product of fermentation with one or more filamentous fungi, from the group of *Monascus*, *Aspergillus*, *Penicillium*, *Pleurotus*, *Pythium*, *Hypomyces*, *Paelicilomyces*, *Eupenicillium*, and *Doratomyces*.
4. Food product according to claim 3, characterized in that the fermented soy ingredient is the product of fermentation with *Monascus*.
5. Food product according to any of claims 1-4, wherein the food product is a spread, meat product, baking good, beverage or bar.
6. Food product according to claim 5, wherein the food product is a bar, beverage or meat product.
7. Food product according to claim 6, wherein the food product is a meat product having the following composition: minced meat 60-80 wt%, mycoprotein 15-25 wt%, 2-10 wt% soy protein comprising statin, 0.1-1 wt% sodium caseinate, 0.1-1 wt% transglutaminase, nitrite salt 0.5-5 wt%, glucose 0.1-1 wt%, *Pediococcus* culture mix 0.001-0.010 wt% 0-1 wt% and optionally other ingredients.

8. Food product according to claim 6, wherein the food product is a meat product having the following composition: 10-40 wt% minced pork meat, 5-20 wt% minced beef meat, 2-10 wt% soy protein comprising statin, 0-5 wt% salt, 0-5 wt% nitrite, 0-5 wt% phosphate, 20-50% water and optionally other ingredients.

EPO - DG 1

09 02 2001

26

(44)

Abstract

A food product suitable for reducing low density lipoprotein cholesterol levels comprising an amount of soy protein of at least 5 grams per average serving and at least 5 mg/kg statins is described. Preferably the food product comprises a fermented soy ingredient.

BEST AVAILABLE COPY

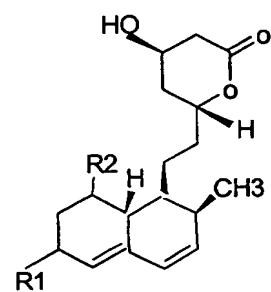
27

EPO - DG 1

09 02 2001

(44)

FIGURE I



BEST AVAILABLE COPY